(0.1mM) stimulated HCO_3^- secretion in intact pancreatic ducts (Venglovecz et al. Gut. 2008). This stimulatory effect of CDC on HCO_3^- secretion was caused by an IP_3-mediated elevation of intracellular calcium concentration and an increase in apical Cl^-/HCO_3^- exchange activity. The aim of this work was to investigate whether CDC could also regulate ion channels in native pancreatic cells. Using standard whole cell current recordings (KCl-rich pipette: NaCl-rich bath solutions) exposure of isolated pancreatic duct cells to CDC (0.1 mM) reversibly increased whole cell currents ~ 3-fold in 75 % of recordings (15/20 cells), and hyperpolarised membrane potential by ~ 18 mV. Resting and activated currents showed marked outward rectification, and were moderately voltage-dependent. CDC-induced currents were inhibited by external barium (5 mM, n=6), as well as by the selective high conductance K^+ channel blocker, iberiotoxin (100nM, n=7). However, they were not sensitive to TRAM34 (selective blocker of intermediate conductance K^+ channels) nor UCL 1684 (selective blocker of small conductance K^+ channels). Bile acid induced-activation was abolished when cytosolic Ca^{2+} buffering was increased with 5.0mM EGTA, and was only moderately reduced by removal of bath Ca^{2+}. Higher concentrations of CDC (>0.5 mM) were deleterious if exposed for prolonged periods. Together these results provide strong evidence that low doses of CDC selectively activate iberiotoxin-sensitive K^+ channels through an increase in cytosolic Ca^{2+}, primarily from internal stores. Activation of a K^+ conductance would hyperpolarise membrane potential and thereby increase the electrochemical driving force for HCO_3^- secretion through electrogenic apical anion exchangers. Supported by The Royal Society.

2759-Pos Board B729

MDCK Monolayers on Silicon Chips - The Epithelial Barrier Function on a Cellular Level

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The MDCK cell line is a general model system for epithelial cells. Insights about the resistance of the trans- and paracellular pathway on a cellular level come from electrical impedance spectroscopy [1]. We now aimed for a direct measurement of the cellular parameters with a variety of techniques to improve theoretical models of the epithelial cell line.(i) The cell-solid distance was determined by fluorescence interference contrast (FLIC) microscopy. (ii) Multi-Transistor-Arrays were used for measuring the thermal fluctuations of extracellular voltage that provide the electrical properties of the distributed resistor-capacitor system, in particular the sheet resistance of the cell-substrate junction. (iii) An alternating voltage is applied to an electrolyte/oxide/silicon capacitor below the cells and phase maps of the transmembrane voltage were measured in three dimensions with a voltage sensitive dye. These maps give resistances and capacitances of a three-dimensional equivalent circuit. In the case of imperfect confluency in comparison to confluent monolayers a surprising shift in distance to the substrate is found. The specific resistance in the junction beneath confluent layers of cells is enhanced. Time resolved measurements with different Na concentrations show an active regulation of the sheet resistance in the junction which can be blocked by inhibition of Na/K/Cl cotransporter. Trans- and paracellular resistances are accessible via transmembrane voltages. Therefore all important parameters on a cellular level can be determined.

[1] C. Lo, C. R. Keese, and I. Giaever, Biophys. J., 69 (1995) 2800

Intracellular Channels

2760-Pos Board B730

Electrophysiological Characterization of Mitochondrial Uncoupling Protein 1

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Uncoupling proteins (UCP1- UCP5) are integral transport proteins of the inner mitochondrial membrane (IMM). They mediate transmembrane ion leak, thus dissipating the electrochemical proton gradient across the IMM and uncoupling mitochondrial respiration and ATP synthesis. UCPs are involved in thermogenesis, reducing fat deposition, and attenuating reactive oxygen species production by mitochondria to protect the cell against oxidative damage and ageing. The mechanism of ion conductance of UCPs has long remained elusive due to the lack of direct methods for measuring ion currents produced by them. To resolve this problem, we applied the patch-clamp technique to the whole inner membrane of mitochondria from the brown adipose tissue to identify and characterize currents produced by the family's founding member, UCP1. In our experiments, both the cytoplasmic and matrix faces of the IMM were exposed to solutions that did not contain any ions normally permeable through ion channels or transporters, except for H^+ and OH^-. Under these conditions, we identified a current that showed all signature properties of UCP1: it was strongly potentiated by micromolar concentrations of unsaturated fatty acids, inhibited by removing endogenous membrane fatty acids with bovine serum albumin, and blocked by micromolar concentrations of purine nucleotides. Moreover, the current was absent in the IMM of the kidney COS-7 cells, in agreement with the fact that UCP1 is specifically expressed in the brown adipose tissue. The transport molecule mediating this current was activated by membrane depolarization and showed robust tail currents upon return to the negative potentials. In a symmetrical pH 5.0 solution the current amplitude was negligible as compared to a symmetrical pH 8.0 solution, suggesting that the current was carried by OH^- but not H^+. Our results indicate that UCP1 is a low-conductance OH^- channel weakly activated by membrane depolarization.

2761-Pos Board B731

TRIC-B Channels Is Critical For Physiological Functions Of Alveolar Epithelial Cells

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Trimeric intracellular cation (TRIC) channels act as counter-ion channels that function in synchronization with Ca^{2+} release from intracellular stores. TRIC channels have two subtypes (TRIC-A and -B) and form homo-trimers with a bullet-like structure. TRIC-B is present in most mammalian tissues, while TRIC-A is expressed in excitable tissues such as brain, heart and muscle. TRIC-A-knockout mice were viable and fertile, whereas TRIC-B-knockout mice showed neonatal lethality. Here we report on TRIC-B-knockout mice, which died within an hour after birth because of acute respiratory failure. TRIC-B-knockout mice exhibited severe cyanosis from appearance observation and respiratory acidosis from blood gas data. In light microscopy analysis, TRIC-B-knockout lung formed immature alveolar spaces and had many glycogen-rich cells. Ultrastructural analysis revealed reduction of type II alveolar epithelial cells (lamellar body positive cells), the number of lamellar body and area of lamellar body in TRIC-B-knockout type II cells. Fluorometric Ca^{2+} measurements showed that Ca^{2+} rise evoked by ATP was significantly reduced in the TRIC-B-knockout type II cells. Moreover, results from ionomycin and cyclopiazonic acid stimulation indicated Ca^{2+} contents in endoplasmic reticulum was significantly increase in TRIC-B-knockout type II cells.

These findings indicate that TRIC-B plays an essential role in glycogenolysis, pulmonary surfactant lipid metabolism, lamellar body biogenesis, maturation of type II cells and surfactant exocytosis. In addition, it is suggested that TRIC-B supported Ca^{2+} release from IP_3R in type II cells, because ATP-evoked Ca^{2+} response reduced in TRIC-B-knockout type II cells.

2762-Pos Board B732

Electrophysiological Comparison of TRIC-A and TRIC-B, Two Trimeric Intracellular Cation Channels Associated with Intracellular Ca2+−Stores

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TRIC membrane proteins have been shown to be present in the endo/sarcoplasmic reticulum (SR) and nuclear membranes of muscle cells and other tissues. TRIC-A has been demonstrated to be a cation-selective channel (Yazawa et al., Nature, 448, 78-82) but the function of TRIC-B has not been determined. We have therefore investigated whether TRIC-B exhibits similar functional properties to TRIC-A by reconstituting purified TRIC-B and TRIC-A into planar phosphatidylethanolamine lipid bilayers under voltage-clamp conditions. We find that TRIC-B also behaves as an ion-channel but with different properties from TRIC-A. Current-voltage relationships of TRIC-B in symmetrical 210 mM KCl or symmetrical 210 mM K-PIPES solutions revealed single-channel conductances of 59 ± 10 pS and 63 ± 5 pS (SD; n=5), respectively, suggesting that TRIC-B is cation selective. In comparison, the conductance of TRIC-A in symmetrical 210 mM KCl or symmetrical 210 mM K-PIPES solutions was 167 ± 26 pS and 158 ± 25 pS (SD; n=3), respectively. The K+ conductance observed with the TRIC-A channel is very similar to that reported by Miller and colleagues for the well known 'SR K+−selective channel' found in skeletal muscle (e.g. Coronado & Miller, J.Gen.Physiol. 79, 529-547). As for the SR K+−channel, we also find that TRIC-A is impermeable to Ca2+, is more open at positive potentials and exhibits a sub-conductance state approximately 50% of the full conductance level. The functional characteristics of TRIC-A and TRIC-B suggest that their localisation on intracellular Ca2+ stores may be vital for the counter-movement of ions to balance transient potential differences generated by Ca2+ movements across the SR membrane.

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